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## Prioritätsbescheinigung über die Einreichung einer Gebrauchsmusteranmeldung

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**Bezeichnung:** Watersoluble prodrugs of propofol

**IPC:** A 61 K 31/05

**Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Gebrauchsmusteranmeldung.**

München, den 6. Mai 2003
**Deutsches Patent- und Markenamt**
**Der Präsident**
Im Auftrag

Wehner

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# WATERSOLUBLE PRODRUGS OF PROPOFOL

## Field of the invention

The present invention relates to esters of propofol (2,6-diisopropylphenol) and propofol derivatives comprising a saccharide with a reducing end group, a method for anesthetizing a mammal as well as a method for treating convulsions, migraine or related diseases, or for inhibition of free radicals in a mammal using said compounds. Furthermore, the present invention relates to said compounds for use as a medicament and the use of said compounds for the preparation of a medicament for anesthetizing a mammal or for treating convulsions, migraine or related diseases, or for inhibition of free radicals in a mammal.

## Background of the invention

Propofol (2,6-diisopropylphenol, see compound 1 of Fig. 1) is an important intravenous agent in the practice of anesthesia. Due to its very low solubility in water, propofol was initially formulated as a 1% w/v solution in the presence of Cremophor EL (a solubilizing surfactant), but the anaphylactic reactions associated with its administration have led to a search for alternative formulations (Trapani G, Altomare C, Sanna E, Biggio G, Liso G. 2000. Propofol in anesthesia. Mechanism of action, structure-activity relationships, and drug delivery. Curr. Med. Chem. 7: 249-271. Franks NP, Lieb WR. 1994. Molecular and cellular mechanisms of general anaesthesia. Nature (Lond). 367: 607-614.). Presently, propofol is formulated in an oil-in-water emulsion (1% w/v) of soya bean oil, glycerol and purified egg phosphatide (Diprivan®, Zeneca UK). Intravenous (i.v.) injection of Diprivan® produces hypnosis rapidly (usually within 40 sec) and smoothly with minimal excitation, but pain at the site of injection is a major adverse effect (Prankerd RD, Stella VJ. 1990. Use of oil-in-water emulsions as a vehicle for parenteral drug administration. J. Parent. Sci. Technol. 44: 139-149.). As a lipid-based emulsion, it suffers from a number of limitations, such as poor physical stability, potential for embolism, and need for strictly aseptic handling (Bennett SN, Mc Neil MM, Bland LA, Arduino MJ, Villarino ME, Perrotta DM. 1995. Postoperative infections traced to contamination of an intravenous anesthetic, propofol. New England Journal of Medicine 333: 147-154.). Moreover, particular care is required in patients with disorders of fat metabolism (Dollery C. (ed.). 1991. Propofol. In Therapeutics

Drugs, Churchill Livingstone, London, Vol 2 pp. 269-271), and the material of the tubes used for infusing the emulsion must be carefully selected.

To avoid these drawbacks, safe alternative dosage forms, in particular aqueous formulations are needed. Approaches in this direction include the complexation of propofol with hydroxypropyl-β-cyclodextrin (Brewster M. 1991. Parenteral safety and applications of 2-hydroxypropyl-β-cyclodextrin. In Duchêne D, editor. New Trends in Cyclodextrins and Derivatives, Paris: Editions de Santé, pp. 313-350. Trapani G, Lopedota A, Franco M, Latrofa A, Liso G. 1996. Effect of 2-hydroxypropyl-β-cyclodextrin on the aqueous solubility of the anaesthetic agent propofol (2,6-diisopropylphenol). Int. J. Pharm. 139: 215-218. Trapani G, Latrofa A, Franco M, Lopedota A, Sanna E, Liso G. 1998. Inclusion complexation of propofol with 2-hydroxypropyl-β-cyclodextrin. Physicochemical, nuclear magnetic resonance spectroscopic studies, and anesthetic properties in rat. J. Pharm. Sci. 87: 514-518.) and chemical delivery systems. The main objectives of these approaches are to increase the hydrosolubility of propofol, improve patient acceptance, e.g. reduced pain at the site of injection, and a decrease in side-effects as well as prolonged action (Pop E, Anderson W, Prokai-Tatrai K, Vlasak J, Brewster ME, Bodor N. 1992. Syntheses and preliminary pharmacological evaluation of some chemical delivery systems of 2,6-diisopropylphenol (propofol). Med Chem. Res. 2: 16-21.). Water-soluble prodrugs of propofol have also been prepared as suitable formulations for parenteral administration (Morimoto BH, Barker PL. Preparation of phosphocholine linked prodrug-derivatives. PCT Int. Appl., 2000, WO 0048572. Stella VJ, Zygmunt JJ, Geog IG, Safadi MS. Water-soluble prodrugs of hindered alcohols or phenols. PCT Int. Appl., 2000, WO 0008033. Sagara Y, Hendler S, Khon-Reiter S, Gillenwater G, Carlo D, Schubert D, Chang J. 1999. Propofol hemisuccinate protects neuronal cells from oxidative injury. J. Neurochem. 73: 2524-2530. Hendler SS, Sanchez RA, Zielinski J. Water-soluble prodrugs of propofol. PCT Int. Appl., 1999, WO 9958555.)

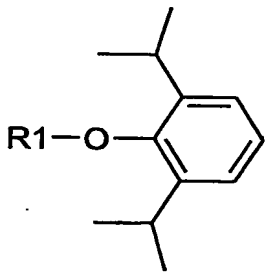
α-Aminoacid ester derivatives of propofol (see compounds 2a-c of Fig. 1) (Trapani G, Latrofa A, Franco M, Lopedota A, Maciocco E, Liso G. 1998. Water-soluble salts of amino acid esters of the anesthetic agent propofol. Int. J. Pharm. 175: 195-204.) were investigated as prodrugs, which demonstrated good aqueous solubility and stability. But the resistance of these compounds against hydrolytic activation in plasma and brain homogenate is much too high for them to actually be considered true prodrugs. Interestingly, some of them were

found to interact with the subtype A of the γ-aminobutyric acid ($GABA_A$) receptor, a major target mediating the pharmacological actions of propofol and other general anesthetics (Trapani G, Altomare C, Sanna E, Biggio G, Liso G. 2000. Propofol in anesthesia. Mechanism of action, structure-activity relationships, and drug delivery. Curr. Med. Chem. 7: 249-271. Franks NP, Lieb WR. 1994. Molecular and cellular mechanisms of general anaesthesia. Nature (Lond). 367: 607-614.). Nevertheless, due to their binding affinity to the $GABA_A$ receptors, similar to that of parent propofol, some of them were suggested as promising candidates for in vivo pharmacological evaluation.

In summary, there is still a need for stable and water soluble propofol derivatives, that are capable of hydrolytic activation under physiological conditions. Specifically, there is a need for stable and water soluble prodrugs of propofol that are readily metabolized to release propofol *in vivo*.

**Disclosure of the invention**

The present invention provides in one aspect compounds having the formula:



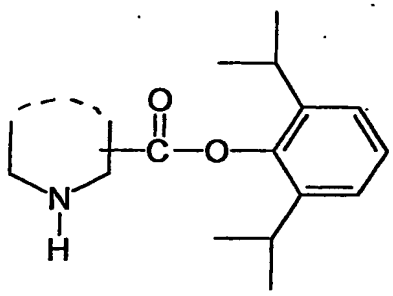

wherein R1 is a cyclic or linear amino acid and their diastereomers or enantiomers in the form of their bases or salts, and wherein the amino acid is optionally further substituted.

According to the present invention the term „amino acid" means any artificial or naturally occurring amino acid characterized by the presence of an amino or imino group and a carboxy group. The term encompasses cyclic and non-cyclic compounds, wherein the cyclic compound may be aromatic or alicyclic. Preferably, the amino acid is a naturally occuring amino acid or a derivative thereof. Preferably, the amino acid is an alpha-, beta-, gamma-, delta- or epsilon-amino acid.

Preferably, the amino acid is C-terminally linked to propofol.

It is preferred that the salts include chloride, sulphate, (hemi)tartrate, (hemi)succinate, (hemi)malate, acetate, lactate and similar anions.

According to a preferred embodiment of the present invention the compounds have the formula:



wherein the heterocyclic group comprises 4 to 5 methylene groups and wherein the heterocyclic group is optionally further substituted.

Preferably, $R_1$ does not comprise a tertiary nitrogen. More preferably, $R_1$ does not comprise a tertiary nitrogen and the compounds of the present invention comprise the above mentioned heterocyclic group comprising 4 to 5 methylene groups and wherein the heterocyclic group is optionally further substituted

It is further preferred that the compounds are cleaved rapidly by esterases.

More preferably, $R_1$ is selected from proline and the three positional isomers of piperidine i.e., pipecolinic, nipecotic, and isonipecotic acid.

It is also more preferred that R1 is selected from the group consisting of tyrosine, tryptophan, phenylalanine or histidine.

The aromatic ring may also be further substituted to create condensed or fused aromatic compounds of the naphthalin, anthracen or phenanthren- type. It is however required that said compounds are essentially water-soluble.

More preferably, said compounds are selected from α-proline, α-pipecolinic acid, ß-nipecotic acid and γ-isonipecotic acid.

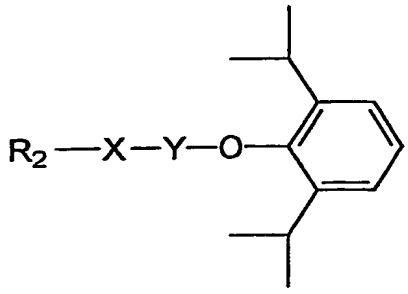
For migraine application the preferred compounds are those which act as depot form i.e. are cleaved more slowly.

In a further preferred embodiment of the present invention, said compounds are selected from α-proline, α-pipecolinic acid, or ß-nipecotic acid, preferably from α-proline or α-pipecolinic acid, and most preferably said compound is α-proline.

The amino acid compound may also be a linear amino acid. Preferably, the amino acid is selected from glycine, alanine, valine, leucine, isoleucine, glutamine, glutamic acid, asparagine, aspartic acid, cysteine, methionine, serine, or threonine.

The skilled person is aware that the amino acid component of propofol derivatives according to the invention is of a basic nature due to the secondary nitrogen atom within the cyclic structure. Therefore, the compounds of the present invention tend to form salts. Preferred salts of the propofol derivatives of the present invention comprise hydrogen and any suitable pharmaceutically acceptable counterion, preferably selected from the group of chloride, sulphate, (hemi)tartrate, (hemi)succinate, (hemi)malate, acetate, lactate and similar anions.

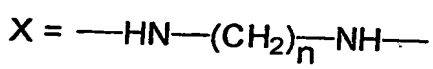
According to another aspect the compounds of the present invention have the formula



wherein $R_2$ denotes a saccharide with a reducing end group, preferably an aldose, and wherein X and Y denote linker groups and wherein the carbohydrate is optionally further substituted.

The linker group may be any linker group known in the art provided that the produced compound is still sufficiently water-soluble. Linker of the hydrazine or glutaric acid type and homologs thereof are preferred.

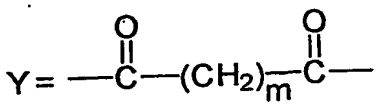
Preferably, X has the formula:



$$X = —HN—(CH_2)_n—NH—$$

wherein n is an integer from 0 to 10, more preferably n = 0.

Preferably, Y has the formula:



$$Y = —\overset{O}{\overset{\|}{C}}—(CH_2)_m—\overset{O}{\overset{\|}{C}}—$$

wherein m is an integer from 0 to 5, more preferably m = 0 or 2.

According to a preferred embodiment $R_2$ is a monosaccharide, disaccharide oligosaccharide or polysaccharide comprising at least one moiety selected from allose, altrose, glucose, mannose, gulose, idose, galactose, talose, sucrose, lactose, maltose, isomaltose, cellobiose, maltobionic acid, and lactobionic acid.

Patricluarly preferred, $R_2$ is maltotriose, lactobionic acid or hydroxyethyl starch.

Also more preferred, $R_2$ comprises up to 40 lactobionic acid moieties, preferably 2 to 7.

Preferably, $R_2$ comprises up to 40 maltobionic moieties, preferably 2 to 7.

Particularly preferred, $R_2$ comprises at least 2 hydroxyethyl glucose moieties wherein the hydroxy ethyl glucose moieties may be substituted. Reference is made to German patent

application DE 10209822.0, the disclosure of which, in particular with respect to the glycosylation pattern of hydroxyethyl starch is incorporated herewith.

In one specific embodiment of the present invention, water-soluble derivatives of propofol are preferably prepared by esterifying the drug with cyclic amino acids, preferably with four specific cyclic aminoacids (compounds 6a-d, see Fig. 1), namely proline and the three positional isomers of piperidine carboxylic acids (i.e., pipecolinic, nipecotic, and isonipecotic acids).

In another specific embodiment, water-soluble derivatives of profolol are obtained by esterifying a saccharide either directly or indirectly via linker groups with propofol. Examples for synthesis are given in the detailed description below.

Their properties such as e.g. solubility, lipophilicity, stability in aqueous solutions, susceptibility to enzymatic hydrolysis in animal plasma and liver, and their ability to interact with $GABA_A$ receptors make them excellent candidate substances for promoting anesthesia and for treating convulsions, migraine or related diseases and for inhibiting free radicals.

Three of the preferred amino acids that were esterified with propofol (with the exception of pipecolinic acid (3b)) are pharmacologically active. (*S*)-Proline is an inibitory amino acid, (*R*)-nipecotic acid is an inhibitor of GABA uptake, and isonipecotic acid is a specific $GABA_A$ agonist (Krogsgaard-Larsen P., Frolund B., Kristiansen U., Frydenvang K., Ebert B. 1977. $GABA_A$ and $GABA_B$ receptor agonists, partial agonists, antagonists, and modulators: design and therapeutic prospects. 5: 355-384). Thus, with the exclusion of propofol pipecolinate (6b), the preferred ester derivatives 6a, 6c and 6d may be considered rather "dual prodrugs", that are converted *in vivo* into two active molecules. Except proline, taken in its natural enantiomeric form (*S*), the other chiral amino acids (i.e., pipecolinic and nipecotic acids) were used in synthesis as racemates. The influence of their steric conformation was not further investigated at this point.

Prolinate derivative 6a is particularly well suited as a water-soluble prodrug. Said compound protects animals against pentylenetetrazole-induced convulsions, and induces an anesthetic action in a short time of a duration that is comparable with that of the marketed propofol emulsion Diprivan®. Its high solubility and stability in water at physiological pH

allow to prepare freeze-dried formulations for parenteral administration. The prolinate derivative 6a is a most preferred embodiment of the present invention.

In a preferred embodiment, the present invention relates to a freeze-dried pharmaceutical composition comprising at least one of the compounds of the present invention, more preferably comprising an α-proline propofol ester.

The susceptibility of the preferred proline ester 6a to enzymatic cleavage by ester hydrolases in plasma and liver affords conversion *in vivo* to the parent drug. Consequently, a 17 mg/mL aqueous solution of proline ester 6a, is equivalent to the commercial oil-in-water emulsion Diprivan® containing 10 mg/mL of propofol.

Prolinate 6a as well as piperidine-2-carboxylate 6b bind as such, i.e. as intact non-hydrolyzed molecules, to the propofol binding site of $GABA_A$ receptors, with $IC_{50}$ values of 30-40 μM (one log unit lower than propofol).

The compounds of the present invention have demonstrated their pharmacological potential in an *in vitro* [35S]TBPS binding assay using rat brain and electrophysiological studies using *Xenopus* oocytes. Moreover, said compounds have demonstrated a pharmacologically effective anticonvulsant and anesthetic activity *in vivo*.

In general, the compounds of the present invention demonstrate high solubility and stability in aqueous solutions and also in physiological media *in vitro*. They are readily hydrolyzed in plasma and liver esterase solutions, many of them even quantitatively within a few minutes.

The compounds of the present invention are also efficacious *in vivo*. Because said compounds readily hydrolize under physiological conditions and release propofol, they are excellent prodrugs for propofol action.

Therefore, the present invention is also directed at a method for anesthetizing a mammal or a method of treating convulsions, migraine or related diseases or for inhibiting in a mammal, wherein a therapeutically effective amount of a compound according to the invention is administered to said mammal.

The term "treating" is intended to refer to all processes, wherein there may be a slowing, interrupting, arresting, or stopping of the progression of a convulsion or convulsions, but does not necessarily indicate a total elimination of all symptoms.

The term "anesthetizing" as used herein is to be understood in the context of the pharmaceutical action of the parent compound propofol.

As used herein, the term "mammal" refers to a warm blooded animal. It is understood that guinea pigs, dogs, cats, rats, mice, horses, cattle, sheep, monkeys, chimpanzees and humans are examples of mammals and within the scope of the meaning of the term. Humans are preferred.

In effecting treatment of a mammal in need of anesthetic treatment or suffering from convulsion, the compounds disclosed by the present invention for said purpose can be administered in any form or mode which makes the therapeutic compound bioavailable in an effective amount, including oral or parenteral routes. For example, products of the present invention can be administered intraperitoneally, intranasally, buccally, topically, orally, subcutaneously, intramuscularly, intravenously, transdermally, rectally, and the like.

Parenteral administration of the compounds of the present invention is preferred.

One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular characteristics of the product selected, the disease or condition to be treated, the stage of the disease or condition, and other relevant circumstances. (Remington's Pharmaceutical Sciences, Mack Publishing Co. (1990)). The products of the present invention can be administered alone or in the form of a pharmaceutical preparation in combination with pharmaceutically acceptable carriers or excipients, the proportion and nature of which are determined by the solubility and chemical properties of the product selected, the chosen route of administration, and standard pharmaceutical practice. For oral application suitable preparations are in the form of tablets, pills, capsules, powders, lozenges, sachets, cachets, suspensions, emulsions, solutions, drops, juices, syrups, while for parenteral, topical and inhalative application suitable forms are solutions, suspensions, easily reconstitutable dry preparations as well as sprays. Compounds according to the invention in a sustained-release substance, in dissolved form

or in a plaster, optionally with the addition of agents promoting penetration of the skin, are suitable percutaneous application preparations. The products of the present invention, while effective themselves, may be formulated and administered in the form of their pharmaceutically acceptable salts, such as acid addition salts or base addition salts, for purposes of stability, modulation of hydrophobicity, increased solubility, and the like.

The amount of active agent to be administered to the patient depends on the patient's weight, on the type of application, symptoms and the severity of the illness. Normally, 0.1 mg/kg to 25 mg/kg of at least one substance of the general formula I is administered, but when applied locally, e.g. intracoronary administration, much lower total doses are also possible.

For practicing the methods of the present invention, said compound of the present invention is preferably administered by all possible routes (intraperitoneal, transdermal, intravenous, intravascular, intramuscular, inhalation), preferred route being as sterile solution for intravenous injection.

Thus, the esters of propofol according to the invention are useful as a medicament. Preferably, said compounds are used for the preparation of a medicament for anesthetizing a mammal or for treating convulsions, for treating migraine or related diseases or for inhibiting free radicals in a mammal.

**Figures**

Fig. 1 shows the structure of propofol (1) and propofol amino acid esters of the prior art (2a-c). A schematic diagram of the preferred method for preparing the compounds of the present invention is depicted in the middle of Fig. 1. The abbreviations used for reagents and substituents are well known to those in the art and explained in example 1. For further details, see example 1. Compounds 6a-d in combination with the substituents a-d at the end of Fig. 1 relate to preferred embodiments of the present invention.

Fig. 2 The plot in Figure 2 shows an $S$ (slope) versus log $k'_w$ plot of the data obtained in the

lipophilicity studies in example 3 and demonstrates that, equal to the polycratic capacity factors, slope values of the H-acceptors 2a-c are smaller than those of the amphiprotics 6a-d, proving the ability of the *S* parameter for encoding the total HB capacity of the compounds.

**Fig. 3** shows the effects of propofol 1 and compounds 6a-d on [$^{35}$S]TBPS binding to unwashed rat cortical membranes. Rat cortical membranes were incubated with 2 nM [$^{35}$S]TBPS for 90 min in the presence of different concentrations of propofol 1, or compounds 6a, 6b, 6c, and 6d. The data is expressed as a percentage of binding measured in the presence of solvent and are means of two experiments.

**Fig. 4** shows the modulatory action of compound 6a (a) and 6d (b) at human $\alpha1\beta2\gamma2$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes. Values are expressed as mean (6-13 different oocytes) ± s.e.m. percentage of the potentiation of the control response to GABA ($EC_{20}$, 2-10 μM).

**Fig. 5** shows the synthesis of activated precursors for use in subsequent synthesis of saccharide-conjugates with propofol.

**Fig. 6** shows the synthesis of saccharide-conjugates with propofol either by direct conjugation (Fig. 6A) or via linker groups (Fig. 6B-D).

The following examples further illustrate the best mode contemplated by the inventors for carrying out their invention. The examples relate to preferred embodiments and are not to be construed to be limiting on the scope of the invention.

**Examples**

Chemicals

Propofol (1, see Fig. 1), dicyclohexylcarbodiimide (DCC), (S)-proline (3a, see Fig. 1), pipecolinic acid (3b, see Fig. 1), nipecotic acid (3c, see Fig. 1), isonipecotic acid (see Fig. 1 3d), and all other reagents were purchased from Sigma-Aldrich (taufkirchen, Germany). Rat

serum (lyophilized powder) and porcine liver esterase (suspension in 3.2 M $(NH_4)_2SO_4$ solution, pH 8) were also purchased from Sigma-Aldrich. Reagents used for the preparation of the buffers were of analytical grade. Fresh deionized water was used in the preparation of all the solutions.

Apparatus

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected. IR spectra were recorded as Nujol films for liquids and KBr pellets for solids on a Perkin-Elmer 283 spectrophotometer. $^1$H-NMR spectra were recorded on a Varian EM 390 spectrometer operating at 90 MHz (Varian, Milan Italy). Chemical shifts are expressed [redacted] values downfield from tetramethylsilane (TMS) used as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c GC-MS low resolution spectrometer (Hewlett-Packard, Milan, Italy) operating in electron impact mode. Elemental analyses were performed on a Hewlett-Packard 185 C, H, N analyzer and agreed with theoretical values to within ± 0.40%. High-performance liquid chromatography (HPLC) analyses were carried out on a Waters Associates Model 600 pump equipped with a Waters 990 variable wavelength UV detector and a 20 µL loop injection valve (Waters, Milan, Italy). HPLC mobile phase was prepared using HPLC-grade methanol. For analysis, a Phenomenex $C_{18}$ column (25 cm x 3.9 mm; 5 µm particles) was used as the stationary phase. A flow rate of 1 mL/min was maintained and the column effluent was monitored continuously at 210 or 270 nm. Quantification of the compounds was carried out by measuring the peak areas in relation to [redacted]se of external standards. Stability studies were carried out at controlled temperature of [redacted] ± 0.2 °C in a water bath.

Animals

Male Sprague-Dawley CD® rats (Charles River, Como, Italy) weighing 180-200 g were used. The animals were kept on a controlled light-dark cycle (light period between 8:00 a.m. and 8:00 p.m.) in a room with constant temperature (22 ± 2°C) and humidity (65%). Upon arrival at the animal facilities there was a minimum of 7 days of acclimation during which the animals had free access to food and water.

Animal care and handling throughout the experimental procedure were performed in accordance with the European Communities Council Directive of 24 November 1986

(86/609/EEC). The experimental protocol were approved by the Animal Ethical Committee of the University of Cagliari.

**Example 1** **Synthesis of cyclic aminoacid esters of propofol**

The propofol esters 6a-d were prepared according to the procedure illustrated in Fig. 1, by reacting the BOC-protected cyclic amino acids 4a-d with propofol 1 in the presence of DCC to give the corresponding esters 5a-d, which when deprotected with HCl gas yielded derivatives 6a-d as hydrochlorides (physical and spectral data of newly synthesized compounds 4a, 5a-d, and 6a-d are shown below in Table I).

[redacted]OC-protected amino acids: preparation of 1-(tert-butoxycarbonyl)proline (4a)

To a stirred mixture of proline (4.60 g, 40 mmol) in $H_2O$ (25 mL) containing triethylamine (8.3 mL, 60 mmol), a solution of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON, 10.58 g, 43 mmol) in acetone (25 mL) was added. Stirring was prolonged for 12 h, and then 125 mL of a mixture of ethyl acetate:water (1:1, v/v) was added. The aqueous phase, combined with water (55 mL), used for washing the organic phase, was further washed with 50 mL of ethyl acetate, and then acidified with cold 0.1 N HCl (pH 2) to give compound 4a as a white precipitate.

[redacted]N-BOC-piperidin carboxylic acids 4b-d were prepared in 87-89% yields, according to the [redacted]ove procedure (analytical data in agreement with those reported in literature (Ho B, Venkatarangan PM, Cruse SF, Hinko C.N, Andersen PH, Crider AM, Adloo AA, Roane DS, Stables JP. 1998. Synthesis of 2-piperidinecarboxylic acid derivatives as potential anticonvulsants. Eur. J. Med. Chem. 33: 23-31. Bonina FP, Arenare L, Palagiano F, Saija A, Nava F, Trombetta D, De Caprariis P. 1998. Synthesis, stability, and pharmacological evaluation of nipecotic acid prodrugs. J. Pharm. Sci. 8: 561-567. Freund R. Mederski WKR. 2000. A convenient synthetic route to spiro[indole-3,4'-piperidin]-2-ones. Helv. Chim. Acta. 83: 1247-1255.).

Esterification of BOC-protected cyclic amino acids: preparation of 2,6-diisopropylphenyl 1-(tert-butoxycarbonyl)pirrolidin-2-carboxylate (5a) as a typical procedure

To a stirred solution of 1 (0.40 g, 2.25 mmol), compound 4a (0.57 g, 2.50 mmol), and dimethylaminopyridine (0.1 g, 0.82 mmol) in dry dichloromethane (15 mL), a solution of DCC (1.4 g, 6.8 mmol) in dry dichloromethane (10 mL) was added dropwise during 10 min. Stirring was continued at room temperature for 24 h, and then the dicyclohexylurea (DCU) precipitate was filtered off. The solution was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica gel (petroleum ether-ethyl acetate 98:2 v/v as eluent) to give compound 5a.

Removal of the tert-butoxycarbonyl group: preparation of 2,6-diisopropylphenyl pirrolidin-2-carboxylate hydrochloride (6a) as a typical procedure

To a stirred and ice-cooled solution of ester 5a (0.50 g, 1.33 mmol) in chloroform (20 mL) HCl gas was bubbled for 5 min. Evaporation of the solvent under reduced pressure gave compound 6a as a white solid.

Table I. Physical and spectral characteristics of newly synthesized compounds.

| Comp. | Yield (%) | Mp (°C) | IR (KBr) (γ max), $cm^{-1}$ |
|---|---|---|---|
| 5a | 72 | 69-71 | 1770,1700 |
| 5b | 68 | 100-104 | 1760,1700 |
| 5c | 70 | 80-84 | 1755,1700 |
| 5d | 67 | 94-97 | 1750,1700 |
| 6a | 90 | 189-192 | 1760 |
| 6b | 87 | 225-228 | 1770 |
| 6c | 95 | 154-156 | 1760 |
| 6d | 90 | 234-236 | 1740 |

**Example 2 Solubility of cyclic aminoacid esters of propofol**

The solubility of the propofol derivatives 6a-d (6b-d as hydrochloride salts) in deionized water at 25 °C was determined by adding excess amount of compound to 1-2 mL of water in screw-capped test tube. The resulting mixture was vortexed for 10 min and then mechanically shaken in a thermostatic bath shaker (100 rpm) for 72 h to attain equilibrium.

Next, the mixture was filtered through a 0.45 μm membrane filter (Millipore®, cellulose acetate) and an aliquot was diluted with an appropriate amount of water and analyzed for the aminoacid ester prodrug content spectrophotometrically at 210 nm. All of the manipulations were made without removal of the test tubes from the water bath, using thermostated pipettes, syringes, and buffer solutions. In Table II, as shown below, solubility data is compared with the data previously determined for propofol derivatives 2a-c (Trapani G, Latrofa A, Franco M, Lopedota A, Maciocco E, Liso G. 1998. Water-soluble salts of amino acid esters of the anesthetic agent propofol. Int. J. Pharm. 175: 195-204.).

Table II. Aqueous solubility, , stability in physiological media, and $GABA_A$ receptor binding of amino acid esters (as hydrochloride salts) of propofol

| Cmp | Solubility (mg/mL) in deionized water[a] | Half-lives at 37 °C[a] | | | [$^{35}$S]TBPS binding $IC_{50}$, μM[e,g] |
|---|---|---|---|---|---|
| | | pH 7.4 buffer | 50% rat serum | Porcine liver esterase (13 U/mL) | |
| 6a | 350.0 | 6 h | 17 min | 17 min | 31.7 |
| 6b | 13.4 | 7 h | 2.5 min | 13 min | 39.5 |
| 6c | 525.0 | [f] | 6 h | 3 h | 152 |
| 6d | 29.7 | [f] | [b,f] | 45 h | [h] |
| 2a | < 0.058[b,c] | [b,f] | | | [b,i] |
| 2b | 0.735[b] | [b,f] | [b,f] | | 6.52[a] |
| [illegible]c | 0.213[b] | [b,f] | [b,f] | | [b,i] |

[a]Data are means of two determination (less than 10% of difference). [(b)]Previously determined in phosphate buffer at pH 7.4. [(c)]Determined as free base.. [(f)]Stable after 48 h. [(g)][$^{35}$S]TBPS binding in unwashed rat brain; for propofol $IC_{50}$ = 4.17 μM. [(h)]No displacement, but a % increase in [$^{35}$S]TBPS binding was observed. [(i)]No displacement.

Compared to propofol, whose solubility under the same conditions was about 0.15 mg/mL, two derivatives, prolinate 6a and nipecotate 6c, their solubilities being 350 and 525 mg/mL, respectively, afforded a strong increase of the aqueous solubility of the anesthetic drug. None of the equations proposed for computing intrinsic solubilities gave accurate predictions (Peterson DL, Yalkowsky SH. 2001. Comparison of two methods for predicting aqueous solubility. J. Chem. Inf. Comput. Sci. 41: 1531-1534. Teitko IV, Yu V, Kasheva TN,

Villa AEP. 2001. Estimation of aqueous solubility of chemical compounds using E-state indices. J. Chem. Inf. Comput. Sci. 41: 1488-1493.), though it was apparent that the most relevant differences in solubility of derivatives 6 could be, at least in part, accounted for by large differences in crystal lattice energies. In fact, the most soluble 6a and 6c have a melting point lower than 200°C whereas pipecolinate 6b and isonipecotate 6d melt at 225 and 234°C, respectively.

**Example 4 Chemical hydrolysis of cyclic aminoacid esters of propofol**

The hydrolysis of the propofol esters 6a-d was studied in aqueous buffer solutions (0.05 M phosphate buffers; ionic strength of 0.5 maintained by adding a calculated amount of KCl) at pH values of 4, 6, and 7.4 and temperature of 37 ± 0.2 °C. The reactions were initiated by adding 100 µl of a stock solution of the ester (13 mg/mL methanol) to 20 mL of the buffer solution preheated at 37 °C, in screw-capped test tubes (final concentration about $2.0 \times 10^{-4}$ M). The solutions were kept in a water bath at a constant temperature, and at appropriate intervals aliquots of 20 µL were withdrawn and analyzed by HPLC. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual propofol ester against time.

**Example 5 Hydrolysis of cyclic aminoacid esters of propofol in physiological solution**

The susceptibility of the derivatives 6a-d to undergo conversion to the parent propofol was studied in 0.05 M phosphate buffer (pH 7.4) containing 50% of rat serum at 37 °C. Each reaction were initiated by adding 100 µL of the methanolic stock solution of compound under examination to 1.6 mL of preheated serum solution (final concentration about $1 \times 10^{-3}$ M) and the mixture was maintained in water bath at 37 °C. At appropriate times, 100 µL samples were withdrawn and added to 500 µL of cold acetonitrile in order to deproteinize the serum. After mixing and centrifugation (10 min at 4000 rpm), 20 µL of the clear supernatant were filtered through 0.2 µm membrane filter (Waters, PTFE 0.2 µm) and analyzed by HPLC.

Hydrolysis of compounds 6a-d in the presence of porcine liver esterase was followed using a reported procedure (Bonina FP, Arenare L, Palagiano F, Saija A, Nava F, Trombetta D,

De Caprariis P. 1998. Synthesis, stability, and pharmacological evaluation of nipecotic acid prodrugs. J. Pharm. Sci. 8: 561-567.).

Results:

The kinetics of hydrolysis of the derivatives 6a-d were determined in 0.05 M phosphate buffers at pHs 4.0, 6.0 and 7.4 at 37°C as well as in rat serum solution and in the presence of porcine liver esterase.

All the examined derivatives were stable at pH values of 4.0 and 6.0 for 48 h, whereas at physiological pH the hydrolysis of prolinate 6a and pipecolinate 6b followed first-order kinetics with half-lives of 6 and 7 h, respectively. The derivatives 6a and 6b, but not 6c and 6d, were found to be cleaved quantitatively to the parent drug in rat serum and porcine liver esterase solutions at 37°C, and the observed half-lives are reported in Table II.

Kinetic data showed that 6a and 6b are stable enough in solution buffered at pH 7.4, their half-lives exceeding 6 h, but undergo a fast cleavage at conditions similar to those prevailing *in vivo*, providing propofol within few minutes. Conversely, compounds 6c and 6d were found to be stable enough both in buffer solution and less susceptible than the α-amino acid esters to esterases' catalysis. The observed high stability toward the chemical hydrolysis can be ascribed to the steric protection of the C(O)O- bond by bulky flanking diisopropyl groups on the phenyl ring. The fact that the proline (6a) and pipecolinic acid (6b) esters, similarly to α-amino acid esters or related short-chained aliphatic amino acid esters (Bundgaard H, Larsen C, Thorbek P.. 1984. Prodrugs as drug delivery systems. XXVI. Preparation and enzymic hydrolysis of various water-soluble amino acid esters of metronidazole. Int. J. Pharm. 18: 67-77.), are less resistant than compounds 6c and 6d to chemical and enzyme-catalyzed hydrolysis could result from either the electron withdrawing effect of the protonated amino group, which activates the ester linkage toward $OH^-$ attack, and (predominantly) the intramolecular catalysis (i.e., intramolecular $N \rightarrow CO$ 1, 2 proton shift) by the neighboring amino group (protonated or not protonated) that promotes ester cleavage. The above finding demonstrates that prolinate 6a is highly soluble, stable in water at physiological pH and rapidly hydrolyzed in plasma. Therefore, compound 6a is an excellent prodrug of propofol for parenteral administration.

**Example 6 *In vitro* [$^{35}$S]TBPS Binding Assay**

Experimental set up:

Rats were killed by decapitation and their brains rapidly removed on ice. The cerebral cortex was dissected out and homogenized in 50 volumes of ice-cold 50 mM Tris-citrate buffer (pH 7.4 at 25°C) containing 100 mM $CaCl_2$ using a Polytron PT 10 (setting 5, for 20 sec) and centrifuged at 20.000 × *g* for 20 min. The resulting pellet was resuspended in 50 volumes of 50 mM Tris-citrate buffer (pH 7.4 at 25°C) and used for the assay. [$^{35}$S]TBPS binding was determined in a final volume of 500 µL consisting of. 200 µL of tissue homogenate (0.20-0.25 mg protein), 50 µL of [$^{35}$S]TBPS (final assay concentration of 1 nM), 50 µl 2 M NaCl, 50 µL of drugs or solvent and buffer to volume. Incubations (25°C) were initiated by addition of tissue and terminated 90 min later by a rapid filtration through glass-fiber filter [redacted]ips (Whatman GF/B, Clifton, NJ), which were rinsed twice with a 4 mL portion of ice-cold [redacted]is-citrate buffer using a Cell Harvester filtration manifold (model M-24m Brandel, Gaithersburg, MD). Filter bound radioactivity was quantitated by liquid scintillation spectrometry. Nonspecific binding was defined as binding in the presence of 100 µM picrotoxin and represented about 10% of total binding. Protein content was determined by the method of Lowry[20] using bovine serum albumin as a standard.

Results:

*Receptor binding.*

$GABA_A$ receptors are sensitive targets for the action of propofol and other general anesthetics (Trapani G, Altomare C, Sanna E, Biggio G, Liso G. 2000. Propofol in anesthesia. Mechanism of action, structure-activity relationships, and drug delivery. Curr. [redacted]d. Chem. 7: 249-271. Franks NP, Lieb WR. 1994. Molecular and cellular mechanisms of general anaesthesia. Nature (Lond). 367: 607-614.). Binding of [$^{35}$S]TBPS, a cage convulsant which binds in close proximity to the chloride channel portion of the $GABA_A$ receptor at level of the picrotoxin binding site, constitutes a tool for studying the function of the $GABA_A$ receptor complex (Squires RF, Casida JE, Richardson M, Saederup E. 1983. [$^{35}$S]t-Butylbicyclophosphorothionate binds with high affinity to brain-specific sites coupled to γ-aminobutyric acid-A and ion recognition sites. Mol. Pharmacol. 23: 326-336). Propofol, mimicking the action of other general anesthetics, such as alphaxalone and pentobarbital (Concas A, Santoro G, Serra M, Sanna E, Biggio G. 1991. Neurochemical action of the general anaethetic propofol on the chloride ion channel coupled with $GABA_A$ receptor. Brain Res. 542: 225-232.), reduces [$^{35}$S]TBPS binding in a concentration-dependent manner.

The ability of the compounds 6a-d to interact with [$^{35}$S]TBPS binding sites was measured and compared with that of propofol. Affinity data, expressed as $IC_{50}$ values (see Table II above), demonstrates that compounds 6a and 6b are able to reduce the [$^{35}$S]TBPS binding, with $IC_{50}$ values one magnitude order higher than $IC_{50}$ value of propofol (4.17 μM). A similar effect, at doses higher than 100 μM, was shown by nipecotate 6c, whereas compound 6d displayed an increase of [$^{35}$S]TBPS binding, an effect similar to that of the antagonist bicuculline (Concas A, Sanna E, Mascia MP, Serra M, Biggio G. 1990. Diazepam enhances bicuculline-induced increase of t-[$^{35}$S]butylbicyclophosphorothionate binding in unwashed membrane preparations from rat cerebral cortex. Neurosci. Lett. 112: 87-91.). Fig. 3 shows the competitive inhibition curves of the examined cyclic amino acid ester derivatives.

**Example 7 Electrophysiological measurements using Xenopus Oocytes**

Experimental set up:

Complementary DNAs encoding the human α1, β2, and γ2 $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (Invitrogen, San Diego, CA). The cDNAs were purified with the Promega Wizard Plus Miniprep DNA Purification System (Madison, WI) and then resuspended in sterile distilled water, divided into portions, and stored at – 20 °C until used for injection. Stage V and VI oocytes were manually isolated from sections of *Xenopus laevis* ovary, placed in modified Barth's saline (MBS) containing 88 mM NaCl, 1 mM KCl, 10 mM Hepes-NaOH buffer (pH 7.5), 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, and 0.33 mM $Ca(NO_3)_2$ and treated with 0.5 mg/mL of collagenase Type IA (Sigma) in collagenase buffer (83 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Hepes-NaOH buffer, pH 7.5) for 10 min at room temperature, to remove the follicular layer. A mixture of $GABA_A$ receptor α1, β2, and γ2 subunit cDNAs (1.5 ng/30 nL) was injected into the oocyte nucleus using a 10 μL glass micropipette (10-15 μm tip diameter). The injected oocytes were cultured at 19 °C in sterile MBS supplemented with streptomycin (10 μg/mL), penicillin (10 U/mL), gentamicin (50 μg/mL), 0.5 mM theophylline, and 2 mM sodium pyruvate. Electrophysiological recordings began approximately 24 h following cDNA injection. Oocytes were placed in a 100-μL rectangular chamber and continuously perfused with MBS solution at a flow rate of 2 mL/min at room temperature. The animal pole of oocytes was impaled with two glass electrodes (0.5 to 3 MΩ) filled with filtered 3 M KCl and the voltage was clamped at -70 mV with an Axoclamp 2-B amplifier (Axon Instruments, Burlingame,

CA). Currents were continuously recorded on a strip-chart recorder. Resting membrane potential usually ranged from -30 to -50 mV. Drugs were perfused for 20 s (7-10 s were required to reach equilibrium in the recording chamber). Intervals of 5 to 10 min were allowed between drug applications.

Results:

Expression of human α1, β2, and γ2 $GABA_A$ receptor subunit constructs in *Xenopus-laevis* oocytes was utilized in a voltage-clamp electrophysiological assay. Figure 4 shows the profiles of prolinate 6a and isonipecotate 6d. Consistent with binding data, GABA-evoked chloride currents elicited at cloned $GABA_A$ receptors were enhanced by 6a and diminished by 6d, both in a concentration-dependent manner with their maximal effects apparent at the concentration of 50 and 100 μM, respectively.

Taken together, the *in vitro* results demonstrated that all the ester derivatives 6a-d modulate $GABA_A$ receptors and possess intrinsic activity, though lower than that of the parent compound 1. Three amino acid esters, 6a-c, behaved like propofol, whereas isonipecotic acid ester revealed a bicuculline-like profile.

**Example 8 *In vivo* screening of anticonvulsant and anesthetic activities**

Experimental set up:

Rats received an intraperitoneal administration of propofol 1 (40 mg/kg, suspended in saline with a drop of Tween 80 per 5 mL) and equimolar doses of compounds 6a and 6d. The anticonvulsant activity against pentylenetetrazole-induced seizures (55 mg/kg) was measured. Rats treated with pentylenetetrazole were considered "protected" when clonic or tonic seizures and death did not occur.

The loss and re-establishment of righting responses, time of anesthesia induction, and sleeping time were also assessed. Rats (five per group) were treated with propofol and Diprivan®, both at a dose of 60 mg/kg, and an equimolar dose of compound 6a (105 mg/kg) and were continuously monitored for the loss of righting reflex (onset and duration). Propofol and its derivative 6a were dissolved in saline with a drop of Tween 80 per 5 mL and administered intraperitoneally in a volume of 0.3 mL per 100 g of body mass. Anesthesia induction (sleep onset) was defined as the time from drug administration to loss

of righting reflex, whereas the sleeping time was the time from the loss of the righting reflex until the animals were plantigrade on all four legs. The significance of differences in behavioral data were analyzed by the ANOVA test.

Results:

Compounds 6a and 6d, displaying *in vitro* agonist and antagonist behavior, respectively, were tested *in vivo* for their anticonvulsant activity, whereas 6a, the derivative showing the best prodrug properties, was compared to propofol, administered either as an oil/water emulsion or as the commercial formulation Diprivan®, for the *in vivo* anesthetic activity. As shown in Table III, compound 6a, like propofol, protected completely the animals from the [redacted]ntylenetetrazole-induced convulsions. In contrast with its *in vitro* $GABA_A$ antagonist [redacted]havior (see Figs 3 and 4), compound 6d appeared to protect animals, though only 60%, against convulsions. Among the hypotheses that may be formulated to explain this result, it may not be excluded that propofol isonipecotate 6d, stable *in vitro* in rat serum solution, can instead be hydrolyzed *in vivo*, releasing propofol and isonipecotic acid (Anderson A, Belleli D, Bennett DJ, Buchanan KJ, Casula A, Cooke A, Feilden H, Gemmel DK, Hamilton NM, Hutchinson EJ, Lambert JJ, Maldment MS, McGuire R, McPhall P, Miller S, Muntoni A, Peters JA, Sansbury FH, Stevenson D, Sundaram H. 2001. α-Amino acid phenolic esters derivatives: novel water-soluble general anesthetic agents which allosterically modulate $GABA_A$ receptors. J. Med Chem. 41: 3582-3591.),[15] a known $GABA_A$ agonist, at anticonvulsant concentrations.

[redacted]e anesthetic activity of compound 6a was investigated by measuring onset and duration of loss of righting reflex, in comparison with that elicited by the clinical propofol formulation (Diprivan®), and an oil/water emulsion of 1 in the presence of Tween 80 (Table III). Induction time of loss of righting reflex subsequent to intraperitoneal administration of compound 1 was notably shorter than that observed for Diprivan®. Compound 6a showed an induction time intermediate between the emulsion formulation and Diprivan®, whereas the duration of anesthesia followed the order propofol emulsion < 6a ≈ Diprivan®. Therefore, compound 6a could be considered an efficacious anesthetic with the same duration of action of Diprivan® but a considerably shorter induction time than the marketed formulation.

Table III. *In vivo* anticonvulsant and anesthetic activities of propofol ester derivatives

| Compounds | Anticonvulsant activity[a] No. of rats protected/ tested | Loss of righting reflex, LRR (sec)[b] Onset | Duration |
|---|---|---|---|
| 1 | 10/10 | 114.4 ± 9.5 | 2245 ± 252 |
| 6a | 10/10 | 162.7 ± 4.3[c, d] | 2403 ± 592 |
| 6d | 6/10 | | |
| Diprivan® | | 289 ± 14.8[c] | 3895 ± 1113 |

[(a)]Protection against clonic and tonic seizures induced by pentylenetetrazole (55 mg/kg, i.p.). Compounds 6a and 6d were tested at doses equimolar to 40 mg/kg propofol. [(b)]Anesthetic activity measured as onset and duration of LRR (mean ± s.e.m.). Compound was administered i.p. at a dose of 105 mg/kg equimolar to 60 mg/kg dose of propofol; $^{(c)}p < 0.01$ vs. propofol-treated animals, $^{(d)}p < 0.01$ vs. Diprivan-treated animals.

**Example 9: Synthesis of Saccharide-conjugates of Propofol**

In a 50 ml round bottom flask 1 ml of propofol was mixed with 2,5 ml TEA at room temperature. When the mixture seemd homogeneous 5,5 mmol of succinnic anhydride were added. The reaction was allowed to proceed under moderate stirring conditions for 22h. The reaction was followed by TLC monitoring or simply observing the disappearance of succinnic anhydride whose solubility in the mixture is low, so most of it remained in the reaction vessel as a white solid. After 22h the reaction was stopped and the solution looked brownish. After elimination of most of the TEA under vacuum, 10 ml of 0,2N HCl were added to the solution which was vigorously stirred and kept in an ice bath for 30min. Thereafter, a white swaying precipitate was removed from the reaction by filtration on a proper funnel filter. The precipitate was dissolved once more in EtOH and was precipitated a second time by adding cold water, filtrated and kept at –20°C.

Three grams of lactobionic acid were dissolved in 5ml of warm DMSO (~70°C). After the complete dissolution 7,5 mmol of mono chloride salt of hydrazine were added to the reaction vessel. The solution was stirred at 45°C for 20h. The proceeding of the hydrazide formation was monitored using TLC coupled with a ninhydrin test to reveal the presence of amino groups. The protonated amine turned yellow in the ninhydrine test. When the reaction was complete, an excess of water and 0,1 N NaOH was added dropwise until a pH~10 was reached. The mixture was frozen and lyophilised. The dry product may be dissolved in water and lyophilised once more to eliminate the last traces of DMSO.

Alternatively, the reaction mixture can be diluted with water, lyophilised and then be incubated overnight on $AgCO_3$ to eliminate the chloride ions. Before making the last lyophilisation a short passage through cation exchanger resins may be run to get rid of possible $Ag^+$ ions.

One mmol of the succinnic acid mono-propofol ester and 370 mg of the lactobionic acid hydrazide were dissolved in 3ml of DMF and stirred at room temperature. A 1:1 molar amount of DCC was added to the solution and the temperature was decreased to 0°C. The reaction was allowed to run one hour under these conditions before switching gradually the temperature to 25°C. The reaction was monitored by TLC coupled with a ninhydrin test. The appearing of the amino functions indicated the end of the reaction (normally after 2h). The reaction was then stopped by adding dilute HCl. The precipitate was washed three times with cold water and then eliminated. The aqueous fractions were frozen and lyophilised. The purity of the product was checked by TLC.

Propofol – Maltotriose prodrug

In 10 ml of a 3:1 DMSO:MeOH mixture 200mg of Propofol were dissolved as well as a three times molar excess of maltotrionic acid, and a catalytic amount of DMAP (dimethylamino pyridine). The solution was left stirring at room temperature for 10 min. In a separate vessel 350 mg of DCC were dissolved in 5ml of the same solution and added to the previous mixture dropwise in a time period of 10min. The reaction was allowed to run under the same conditions for 20h and was then stopped and filtrated. The coupling product was recovered by precipitation in acetone (50 ml) and washed several times with EtOH (100 ml), AcOEt (100 ml) and finally acetone (100 ml). The reaction was monitored by TLC and the purity of the product was also confirmed by RP–HPLC on a C–18 column.

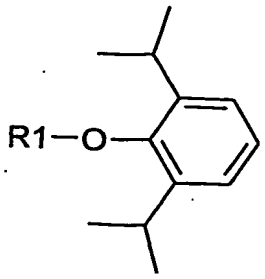
Propofol – oxHES10kD prodrug

In 10 ml of a 5:1 DMSO:MeOH mixture 200mg of Propofol were dissolved as well as a three times molar excess of oxHES10kD, and a catalytic amount of dimethylamino pyridine. The solution was left stirring at the temperature of 40°C. In a separate vessel 350 mg of DCC were dissolved in 5 ml of the same solution and added to the previous mixture dropwise in a time period of 10 min. The reaction was allowed to run under the same conditions for 30h

and then stopped and filtrated. The coupling product was recovered by precipitation in acetone (50 ml) and washed several times with MeOH (100 ml), AcOEt (100 ml) and finally acetone (100ml). The reaction was monitored by TLC and the purity of the product was confirmed by RP–HPLC on a C–18 column.

Claims:

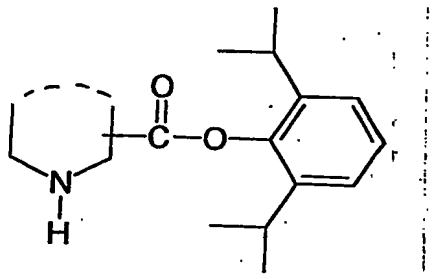
1. Compound having the formula:




wherein R1 is a cyclic or linear amino acid and their diastereomers or enantiomers in the form of their bases or salts, and wherein the amino acid is optionally further substituted.

2. Compound according to claim 1, wherein the amino acid is C-terminally linked to propofol.

3. Compound according to claim 1 or 2 having the formula




wherein the heterocyclic group comprises 4 to 5 methylene groups and wherein the heterocyclic group is optionally further substituted.

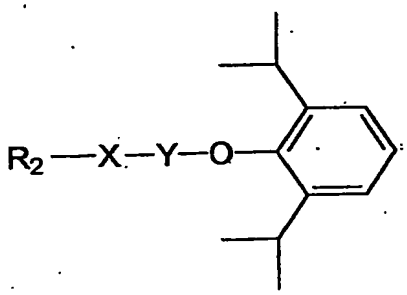
4. Compound according to claim 3, wherein the amino acid component is selected from proline, pipecolinic acid, nipecotic acid and isonipecotic acid.

5. Compound according to claim 4, wherein the amino acid component is selected from α-proline, α-pipecolinic acid, ß-nipecotic acid and γ-isonipecotic acid.

6. Compound according to claim 5, wherein the amino acid component is selected from α-proline, α-pipecolinic acid, or ß-nipecotic acid, preferably from α-proline or α-pipecolinic acid, and most preferably is α-proline.

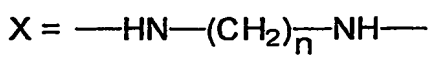
7. Compound according to claim 1 wherein the amino acid is selected from glycine, alanine, valine, leucine, isoleucine, glutamine, glutamic acid, asparagine, aspartic acid, cysteine, methionine, serine, threonine

8. Compound having the formula

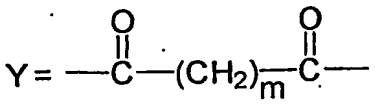


wherein $R_2$ denotes a saccharide with a reducing end group, preferably an aldose, and wherein X and Y denote linker groups and wherein the carbohydrate is optionally further substituted.

9. Compound according to claim 8, wherein



and n= 0 to 10, preferably n = 0, and



and m= 0 to 5, preferably m = 0 or 2

10. Compound according to claim 8 or 9, wherein $R_2$ is a monosaccharide, disaccharide oligosaccharide or polysaccharide comprising at least one moiety selected from allose,

altrose, glucose, mannose, gulose, idose, galactose, talose, sucrose, lactose, maltose, isomaltose, cellobiose, maltobionic acid, and lactobionic acid.

11. Compound according to claim 10 wherein $R_2$ is maltotriose, lactobionic acid or hydroxyethyl starch.

12. Compound according to claim 11, wherein $R_2$ comprises up to 40 lactobionic acid moieties, preferably 2 to 7.

13. Compound according to claim 11, wherein $R_2$ comprises up to 40 maltobionic [redacted]ieties, preferably 2 to 7.

14. Compound according to claim 11, wherein $R_2$ comprises at least 2 hydroxyethyl glucose moieties wherein the hydroxy ethyl glucose moieties may be substituted.

15. A method for anesthetizing a mammal, wherein a therapeutically effective amount of a compound according to any one of claims 1 to 14 is administered to said mammal.

16. A method of treating convulsions in a mammal, migraine or for inhibiting free radicals, wherein a therapeutically effective amount of a compound according to any one of claims 1 to 14 is administered to said mammal.

[redacted]. A compound according to any of claims 1 to 14 for use as a medicament.

18. Use of a compound according to any of claims 1 to 14 for the preparation of a medicament for anesthetizing a mammal.

19. Use of a compound according to any of claims 1 to 14 for the preparation of a medicament for treating convulsions, migraine or for inhibiting free radicals in a mammal.

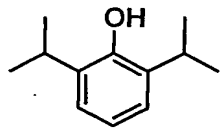
20. A pharmaceutical composition comprising at least one of the compounds according to any one of claims 1 to 14, more preferably comprising an α-proline propofol ester.

21. Freeze-dried pharmaceutical composition comprising at least one of the compounds according to any one of claims 1 to 14, more preferably comprising an α-proline propofol ester.

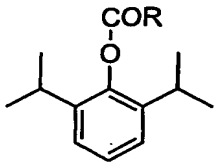
## Abstract

The present invention relates to esters of propofol (2,6-diisopropylphenol) and propofol derivatives comprising a saccharide with a reducing end group, a method for anesthetizing a mammal as well as a method for treating convulsions, migraine or related diseases or for inhibiting free radicals in a mammal using said compounds. Furthermore, the present invention relates to said compounds for use as a medicament and the use of said compounds for the preparation of a medicament for anesthetizing a mammal or for treating convulsions, migraine or related diseases or for inhibiting free radicals in a mammal.

Fig. 1

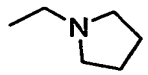


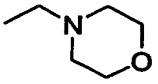

1

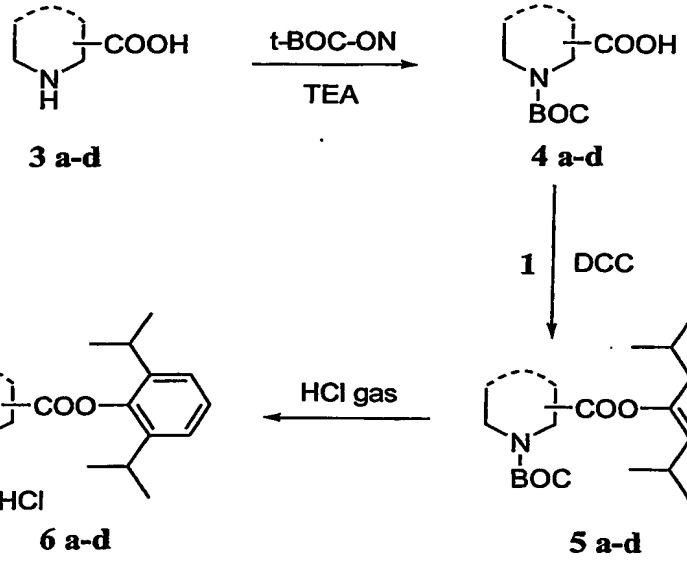


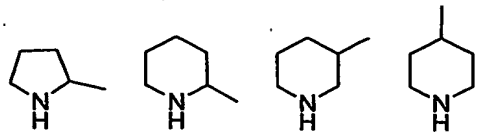

2 a-c

a, R = 


b, R = 


c, R = 


a b c d

**Fig. 2**

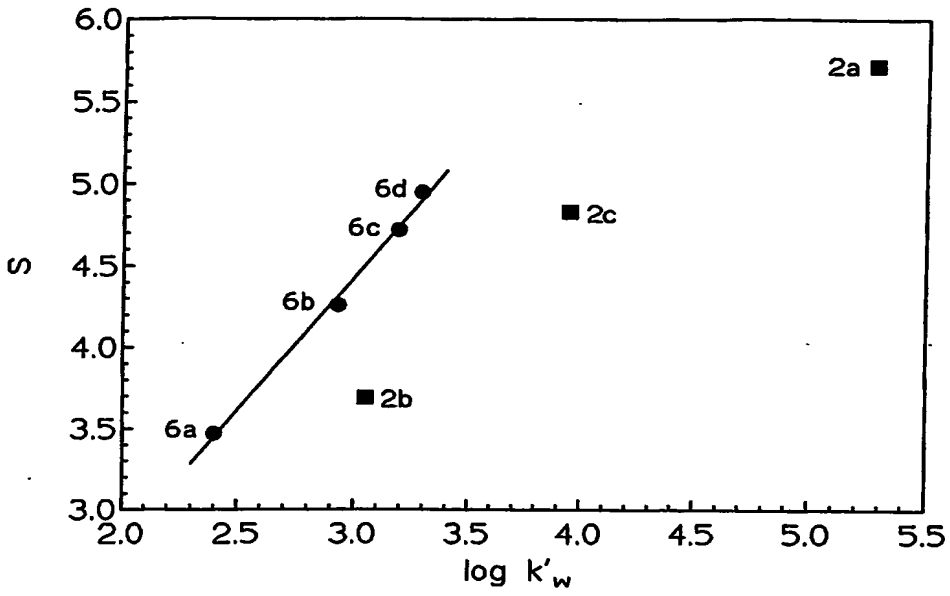

6.0
5.5
5.0
4.5
4.0
3.5
3.0
S
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
log k'w
2a
2c
2b
6a
6b
6c
6d

**Fig. 3**

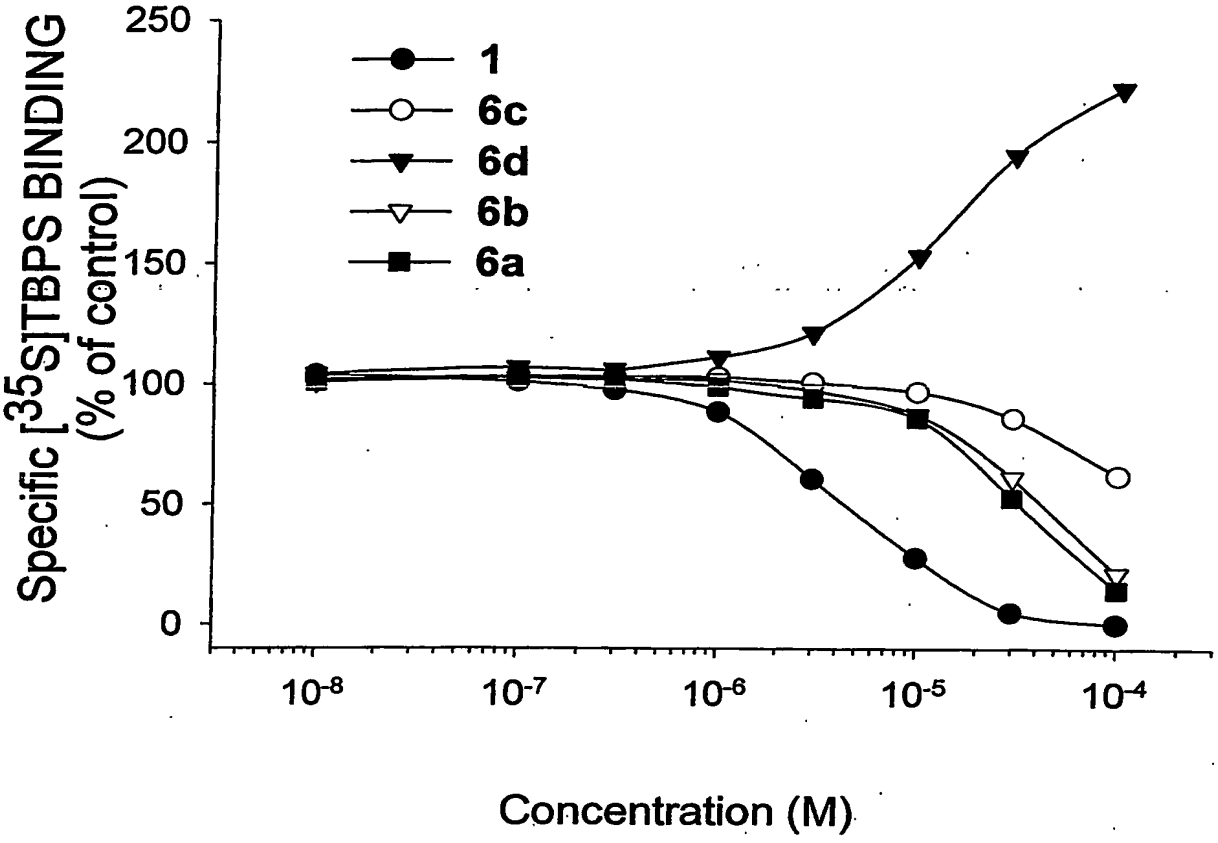

Specific [35S]TBPS BINDING
(% of control)
250
200
150
100
50
0
1
6c
6d
6b
6a
10-8
10-7
10-6
10-5
10-4
Concentration (M)

Fig. 4(a)

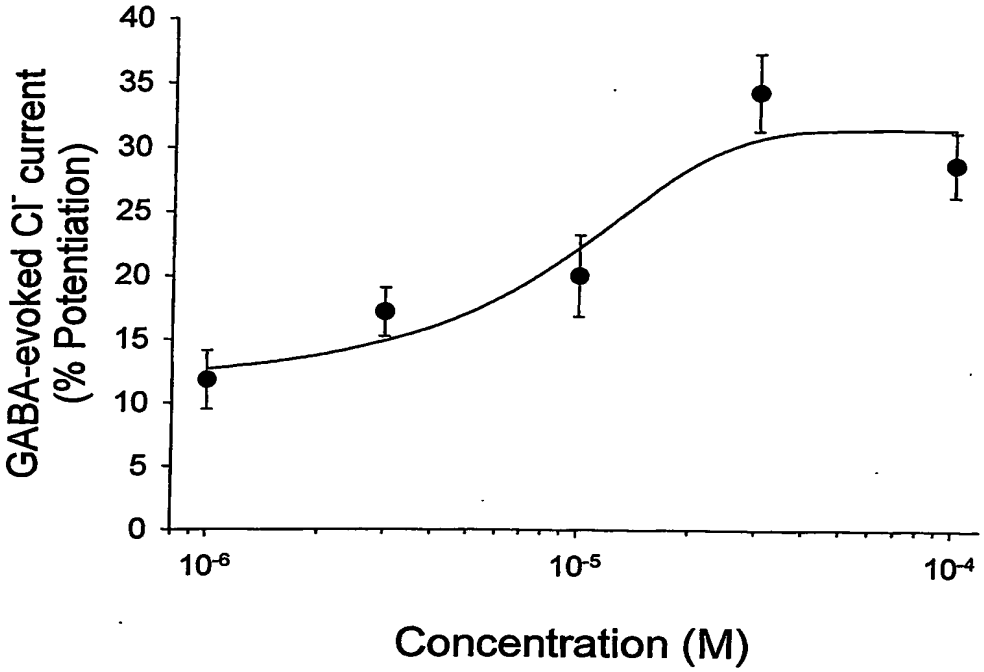
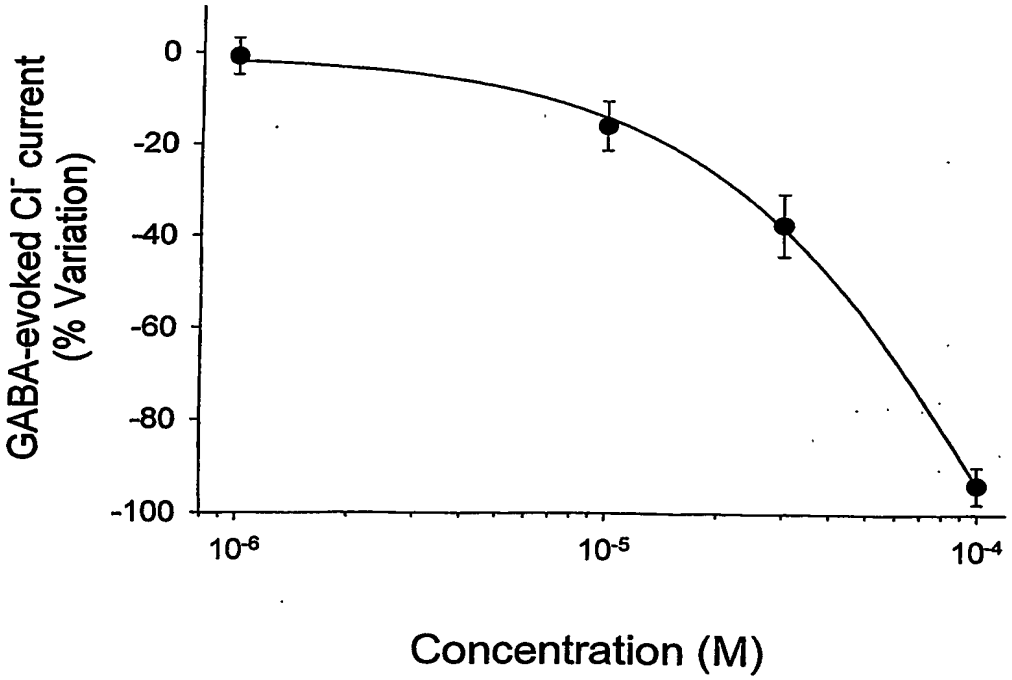

GABA-evoked Cl⁻ current
(% Potentiation)
40
35
30
25
20
15
10
5
0
10-6
10-5
10-4
Concentration (M)

Fig. 4(b)


GABA-evoked Cl⁻ current
(% Variation)
0
-20
-40
-60
-80
-100
10-6
10-5
10-4
Concentration (M)

# Fig. 5A

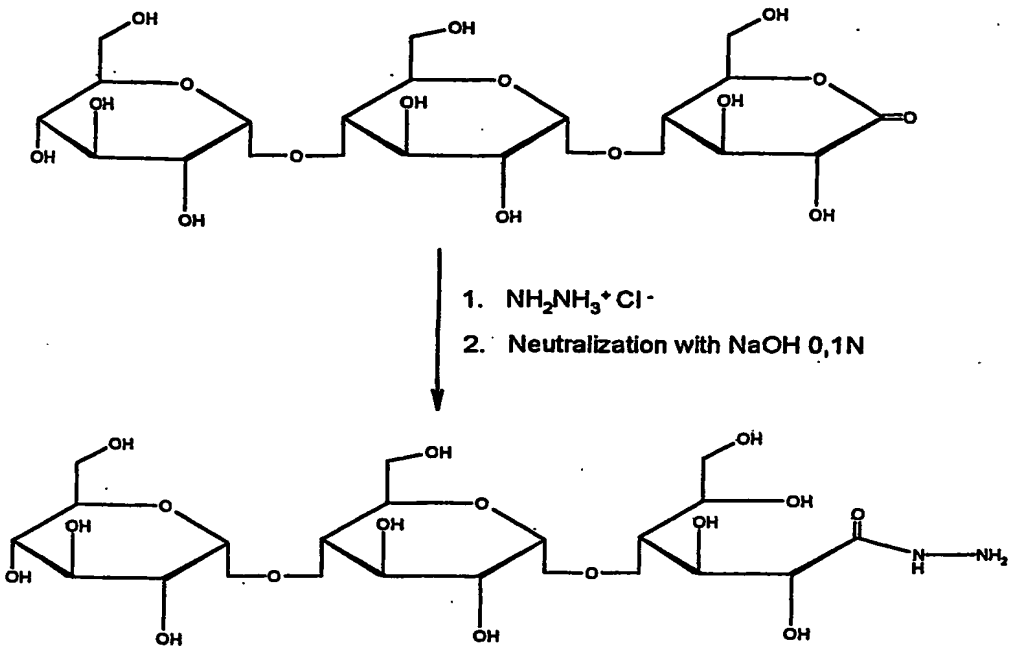
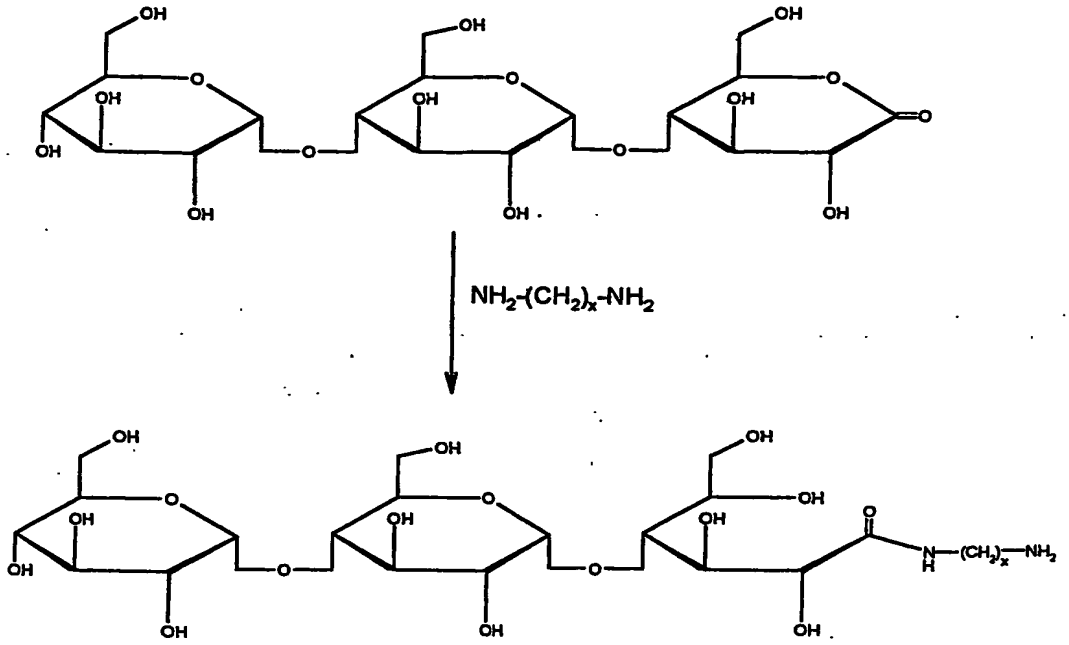
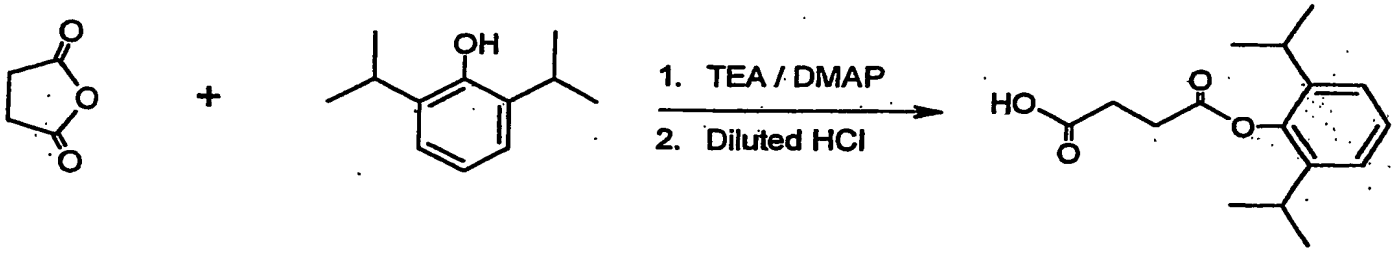


Fig. 5B

1.




2.



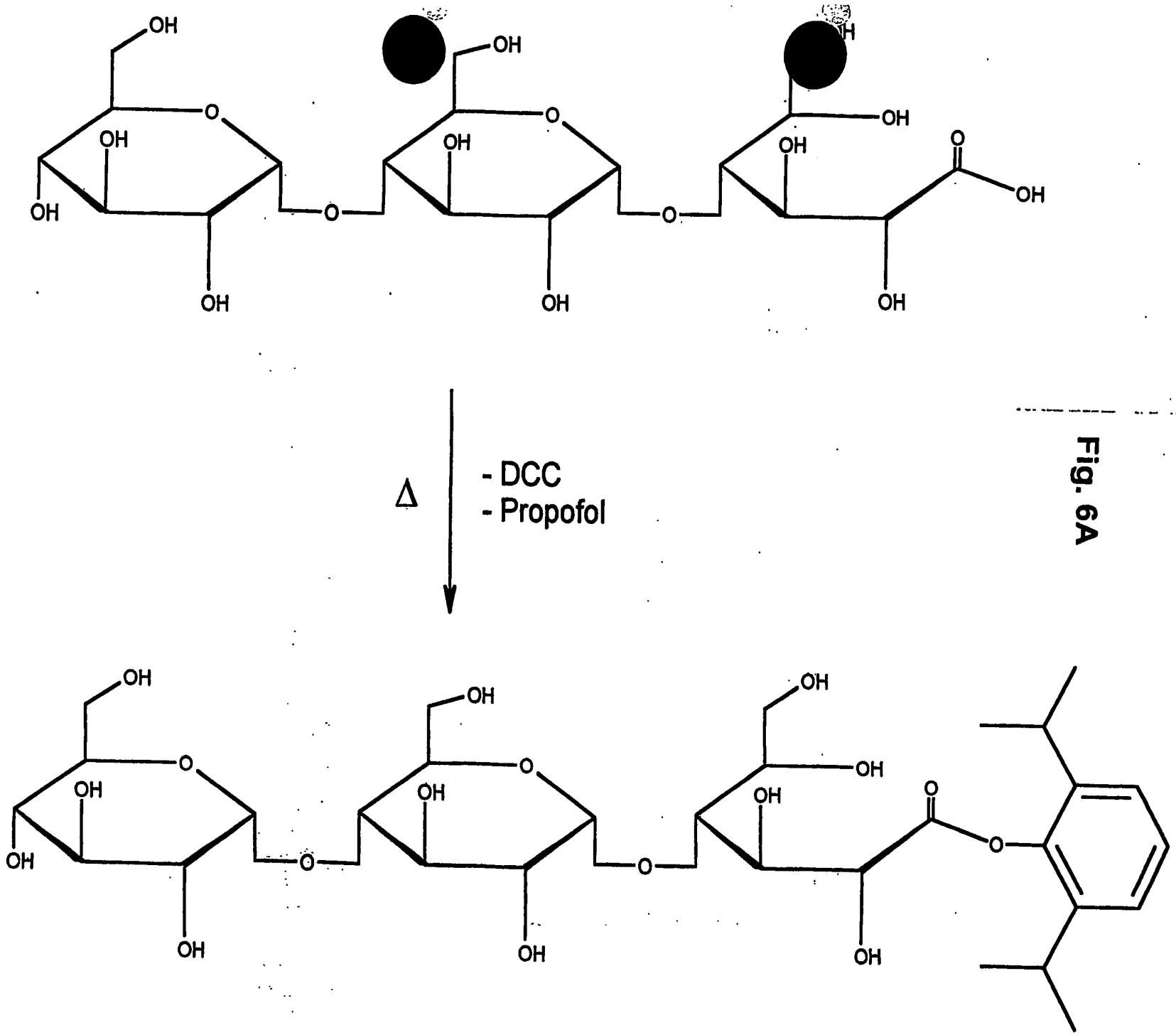

OH
O
OH
OH
OH
O
OH
OH
O
OH
OH
OH
O
OH
Δ
- DCC
- Propofol
OH
O
OH
OH
OH
O
OH
OH
OH
O
OH
OH
O
O

Fig. 6A

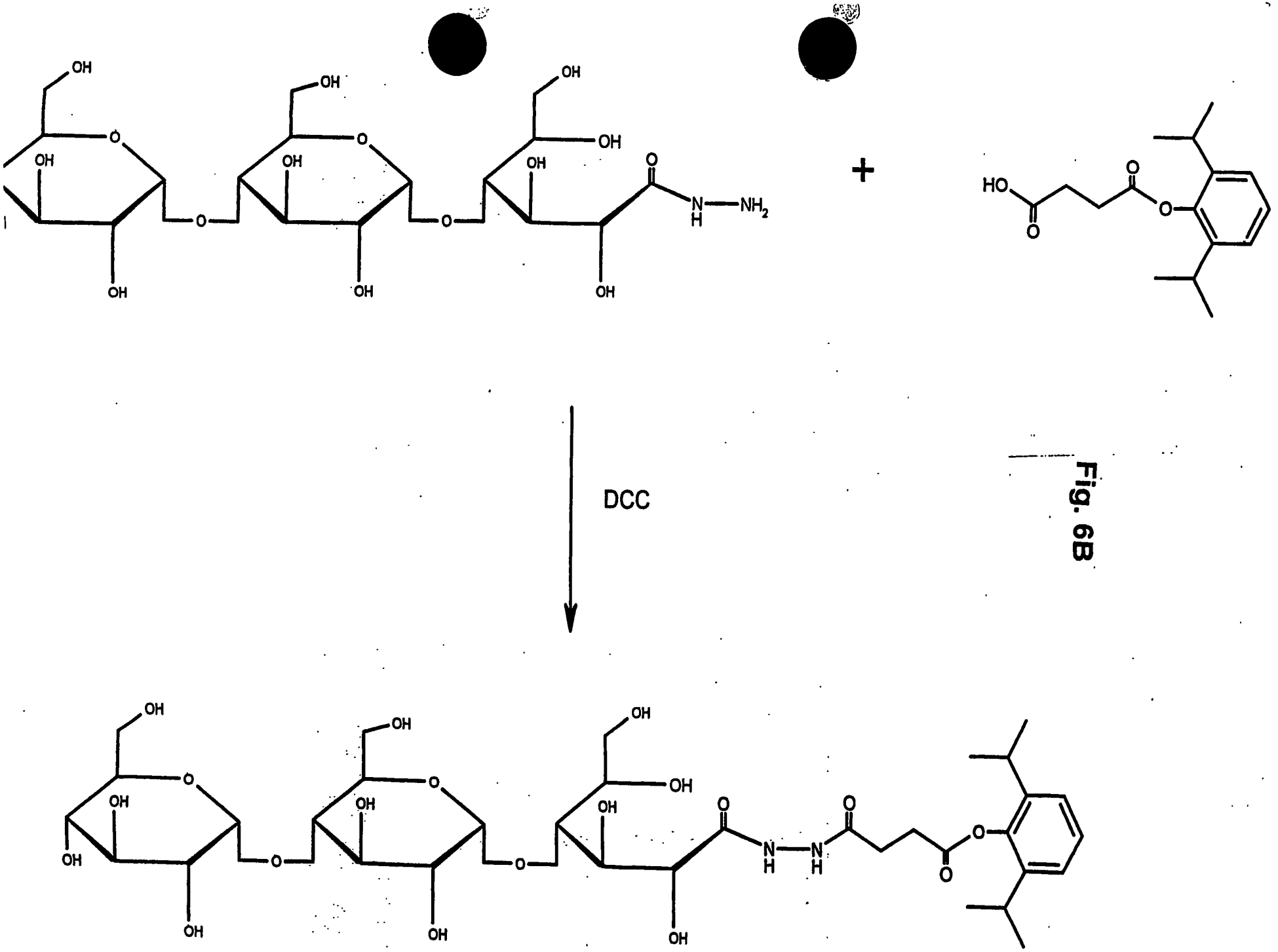



Fig. 6B

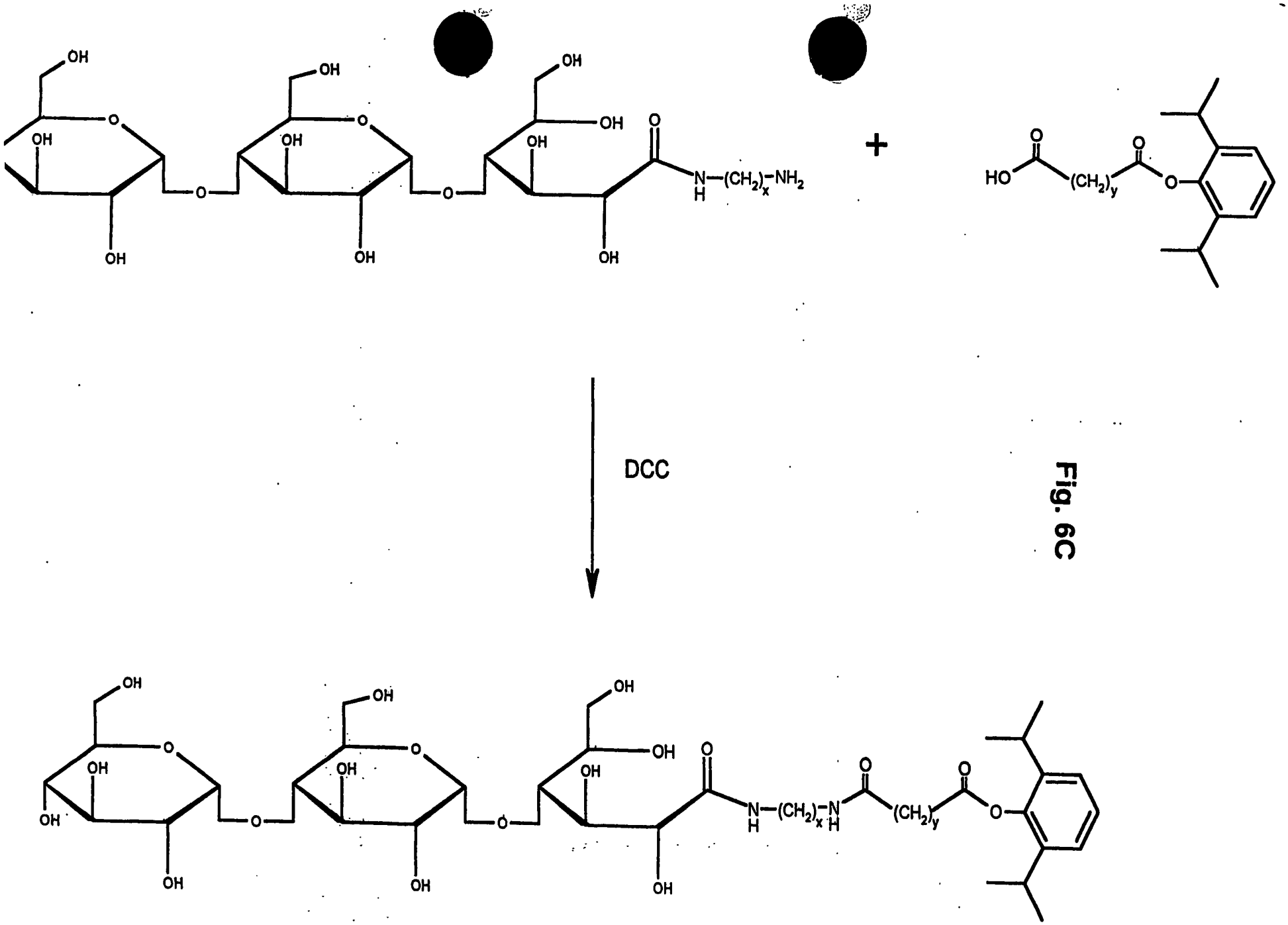



Fig. 6C

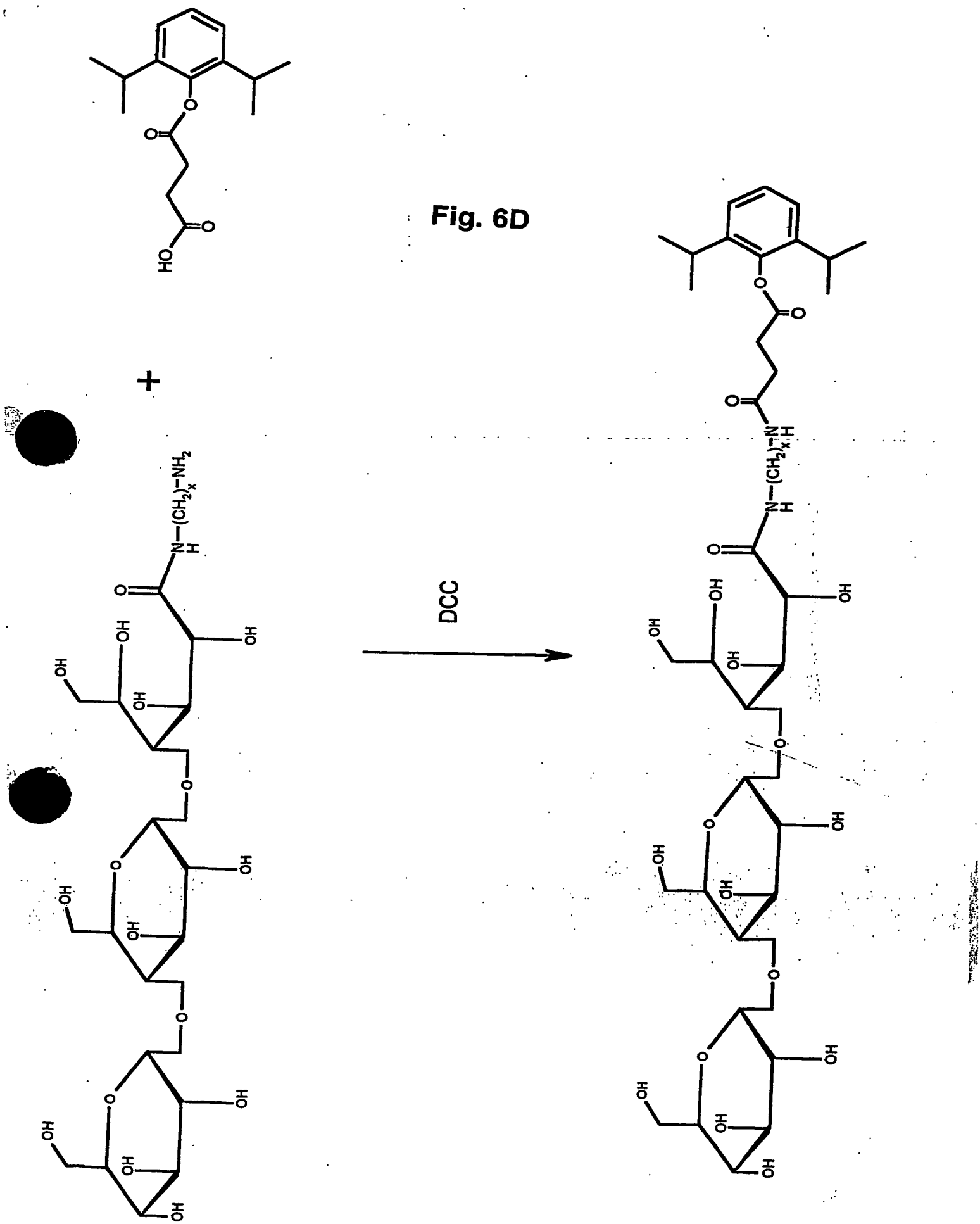



Fig. 6D